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A New Species of *Glyphoglossus* Günther, 1869 (Anura: Microhylidae) from Western Yunnan, China

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Abstract A new species of microhylid frog of the genus *Glyphoglossus* Günther, 1869 is described from Huadianba, Cangshan Mountain, Dali Bai Autonomous Prefecture, Yunnan Province, China. *Glyphoglossus huadianensis*, new species, is compared with congeners from China and other parts of Southeast Asia, and was distinguished from the others by the following combination of characters: adult male body size up to 37.8 mm; pupil rounded; supratympanic fold distinct; tympanum concealed; toe tips obtuse; subarticular tubercles prominent and rounded; foot webbing extensive; outer metatarsal tubercle present; dorsum tuberculate, yellowish-brown/earth-yellow; and a pair of conspicuous large round spots in groin. The new species is the tenth species of *Glyphoglossus* to be described, and the second known from China.

Keywords *Glyphoglossus huadianensis* **sp. n.**, taxonomy, morphology, China

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1. Introduction

Currently, nine nominal species, which are known from Southeast Asia (Frost, 2020), are recognized in the microhylid genus *Glyphoglossus* Günther, 1869. Only one species is known in China, *Glyphoglossus yunnanensis* (Boulenger, 1919). Owing to rare collection, little is known for most species except *Glyphoglossus molossus* Günther, 1869, *Glyphoglossus guttulatus* (Blyth, 1856) and *Glyphoglossus yunnanensis*. The systematics of *Glyphoglossus* were once very confusing for above reason, and several junior synonyms are on record: *Calluella* Stoliczka 1872, *Colpoglossus* Boulenger 1905, *Dyscophina* van Kampen 1905, and *Calliglutus* Barbour and Noble 1916 (Frost, 2020). The genus *Glyphoglossus* was designated to place *Glyphoglossus molossus*, which was originally unassigned to any subfamily of Microhylidae (Frost *et al*., 2006), and was subsequently transferred to Microhylinae by Van Bocxlaer *et al*. (2006). Matsui *et al*. (2011) reported *Calluella* Stoliczka, 1872, to be paraphyletic with respect to *Glyphoglossus* based on mtDNA phylogenetic analysis. Meanwhile, a sister-relationship of *Glyphoglossus* with *Calluella* was also suggested by Pyron and Wiens (2011). Based on denser samplings of *Calluella*, Das *et al*. (2014) confirmed the placement of *Glyphoglossus* within *Calluella*. According to the law of priority, *Calluella* Stoliczka, 1872 was recognized as a junior synonym of *Glyphoglossus* by Peloso *et al*. (2016).

Based on extensive sampling and in-depth investigation into the phylogeographic structure of *G. yunnanensis*, Zhang *et al*.

(2020) discovered there were four main maternal lineages in this nominal species on base of 2154 bp concatenated mitochondrial gene fragments, one of which (lineage LD, Figure 1) occurs in western Yunnan, China and is deeply separated from the remaining lineages with an average divergence of 9.3% (LD/E, 9.6%; LD/W, 9.1%; and LD/C, 9.2%.) (Figure 1). Lineage LD was also shown to be differentiated from other known congeners (Figure 1).

Here we further investigate the identity of lineage LD and describe it as a new species. Morphological comparisons demonstrate that the new species is distinctive from *G. yunnanensis* and other known congeners and therefore warrants taxonomic recognition.

2. Materials and Methods

2.1. Sampling Seven specimens were collected in Dali Bai Autonomous Prefecture, Yunnan, China (25°52'05" N, 100°01'05" E) in May, 2010, and six in Lijiang, Yunnan, China (26°51'18" N, 100°13'40" E) in June, 2010. Following Animal Use Protocols approved by the Kunming Institute of Zoology Animal Care and Ethics Committee, specimens were photographed in the field prior to euthanasia, and then fixed in 90% ethanol before being stored in 70% ethanol. A portion of liver or muscle tissues were preserved in 99% ethanol. Sexes of the type specimens were determined through checking vocal sac openings. Specimens and tissue samples were deposited at Kunming Institute of Zoology, Chinese Academy of Sciences (KIZA00252–KIZA00258, KIZ 2014005754, KIZ 2014005781–KIZ 2014005785).

2.2. Genetic distance Pairwise genetic distances (Kimura 2-parameter) among species of *Glyphoglossus* were calculated using MEGA 7 (Kumar *et al.*, 2016) based on a concatenated data of 16S rRNA, CO1, and CYTB of Zhang *et al.* (2020). The GenBank accession numbers of specimen used in this study were compiled in Table S1.

2.3. Morphology The morphological measurements were taken to the nearest 0.1 mm with digital calipers. Comparative data on the morphology and taxonomy of *Glyphoglossus* were obtained from previous publications: Barbour and Noble (1916), Blyth (1856), Boulenger (1904; 1919), Das *et al.* (2004), Das *et al.* (2014), Fei *et al.* (1999), Günther (1869), Inger (1966), Kiew (1984), Liu (1950), Manthey and Grossmann (1997), Parker (1934), and Van Kampen (1905).

Morphylogical terminology followed Das *et al.* (2004) and Das *et al.* (2014). Abbreviations used for measurements (in mm) and meristic characters are as follows: Snout-vent length (SVL, from tip of snout to vent); tibia length (TBL, distance between surface of knee and surface of heel, with both tibia and tarsus flexed); head length (HL, distance from tip of snout to angle of jaws); head width (HW, measured at angle of jaws); head depth (HD, greatest transverse depth of head, taken posterior of the orbital region); eye diameter (ED, horizontal diameter of the eyes); interorbital distance (IO, least distance between upper eyelids); internarial distance (IN, distance between nostrils); eye to snout distance (E-S, distance between anterior-most point of eyes and tip of snout); eye to nostril distance (E-N, distance between anterior-most point of eyes and nostrils); eye to tympanum distance (E-T, distance between posterior-most point of eye and anterior edge of tympanum); axilla to groin distance (A-G, distance between posterior edge of forelimb at its insertion to body to anterior edge of hindlimb at its insertion to body); body width (BW, greatest width of body); tympanum diameter (TD, vertical and horizontal); width of upper eyelid

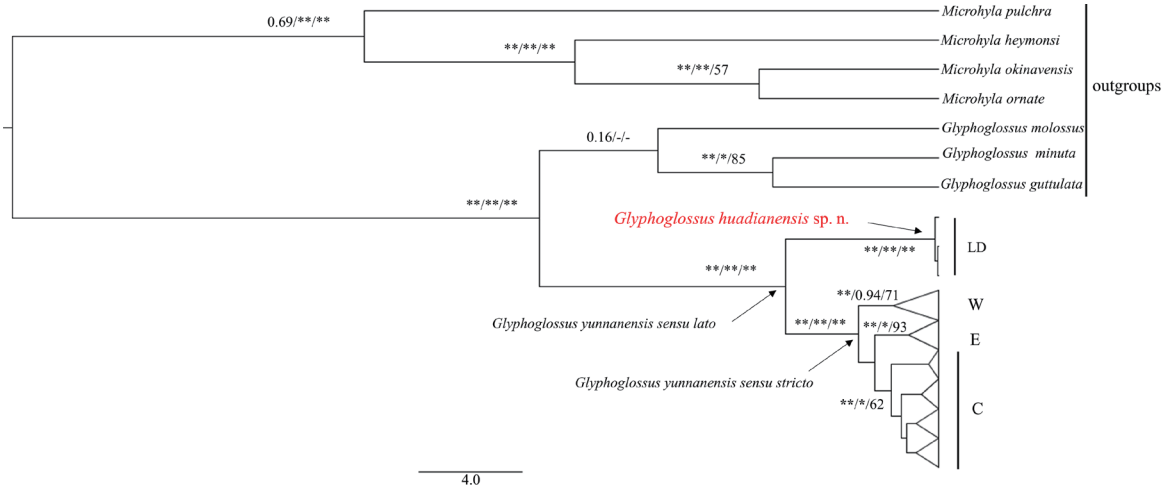

Figure 1 Simplified Bayesian inference (BI) tree of the species *G. yunnanensis sensu lato* reproduced from Zhang *et al.* (2020). Posterior probabilities (BEAST), Bayesian Posterior Probabilities (BPP), and maximum parsimony (MP) bootstrap values (* > 95%, ** > 99%) are shown for main clades. Vertical bars show clade assignment.

(UE, greatest width of upper eyelid). Webbing formula notation follows Savage and Heyer (1967) as modified by Myers and Duellman (1982).

With a correlation matrix of size-standardized measurements (all measurements divided by SVL), a multivariate principal component analysis (PCA) was performed. Scatter plots of the first two principal components scores were used to investigate the differences between the new species and *G. yunnanensis*. The significance of differences between the two species for sizecorrected values of measurements was tested by means of one-way ANOVA ($P < 0.01$ as significant). Data were firstly tested for normality (using Shapiro-Wilk Test) and homogeneity (using Levene's test). All analysis above mentioned were conducted in IBM SPSS Statistics 24 (IBM Corp).

3. Results

3.1. Genetic divergence Lineage LD was deeply separated from *G. yunnanensis sensu stricto* (lineage W+C+E) with a divergence of 8.9%, and from other known congeners 7.8%–20.0% (Table 1).

Table 1 Estimates of Net Evolutionary Divergence between *Glyphoglossus huadianensis* **sp. n.** and other known congeners.

	Taxon	1	2	3	4	5
1	*G. yunnanensis sensu stricto* (lineage W+C+E)					
2	*G. huadianensis* **sp.n.** (lineage LD)	0.089				
3	*G. minuta*	0.078	0.081			
4	*G. guttulata*	0.142	0.183	0.081		
5	*G. molossus*	0.139	0.200	0.084	0.146	

3.2. Morphometric analysis Morphometric data of the new species and *G. yunnanensis* are summarized in Table 2. The first two principal components were retained, which had eigenvalues above 1.0 and cumulative contribution of variance accounted to 58.14% (Table 3). Loadings for PC1, which accounted for 46.95% of the total variance, were most heavily loaded (load factor > 0.70) on HL, HD, TD, E-S, E-N, IO, and HW (Table 3). Differentiation was found along the PC 1 axis between *G. yunnanensis* and the new species (Figure 2). The second principal component (PC 2) accounted for 11.19% of the total variance (Table 3), but no clear separation was observed

Table 2 Measurements (in mm) of *G. huadianensis* **sp. n.** and *G. yunnanensis*. The specimens are all males. Abbreviations defined in text.

species	Voucher No.	SVL	TBL	HL	HW	HD	ED	IO	IN	E-S	E-N	E-T	A-G	BW	TD	UE
Glyphoglossus huadianensis **sp. n.** (lineage LD)	KIZ 2014005754	37.2	15.7	8.8	12.8	6.7	3.7	3.1	2.5	3.7	2.2	1.2	17.8	22.3	2.6	2.1
	KIZ 2014005781	34.8	15.5	7.9	11.8	6.9	3.7	2.7	2.4	3.5	2.0	1.0	16.5	20.6	2.3	2.2
	KIZ 2014005782	37.3	15.5	8.8	12.9	7.0	3.7	2.9	2.6	3.6	2.2	1.2	17.4	20.1	2.4	2.3
	KIZ 2014005783	36.9	16.4	8.5	12.2	7.3	3.4	3.0	2.4	3.2	2.2	1.4	18.9	22.4	2.1	2.1
	KIZ 2014005784	35.8	15.3	8.6	12.8	6.8	3.9	3.1	2.5	3.2	2.0	1.0	16.6	22.7	1.7	2.0
	KIZ 2014005785	37.5	15.5	8.6	12.2	6.7	4.0	3.1	2.5	3.5	2.1	1.3	17.4	20.4	2.1	2.2
	KIAZ00252	36.0	15.0	8.5	11.8	6.3	3.3	3.0	2.4	3.3	2.0	1.3	15.4	18.7	2.4	2.0
	KIZA00253	35.8	15.1	9.0	12.8	6.8	3.7	2.8	2.3	3.6	2.1	1.2	15.3	21.5	2.2	2.3
	KIZA00254	37.3	15.7	9.2	13.1	6.4	3.5	2.9	2.3	3.5	2.2	1.2	15.5	20.6	2.3	2.3
	KIZA00255	36.1	15.4	8.8	12.5	6.7	3.6	2.9	2.3	3.3	2.1	1.1	15.1	18.5	2.5	2.1
	KIZA00256	36.5	15.4	9.2	12.8	6.8	3.7	3.0	2.7	3.9	2.4	1.1	15.3	20.5	2.1	1.9
	KIZA00257	35.9	15.7	8.8	12.6	6.9	3.4	3.0	2.3	3.3	2.1	1.0	16.4	18.8	2.3	2.2
	KIZA00258	37.8	15.7	9.3	13.0	7.1	3.6	2.8	2.5	3.5	2.3	0.9	15.5	19.6	2.4	2.1
Glyphoglossus yunnanensis (lineage W+C+E)	KIZ 2013000259	32.7	14.0	9.5	12.2	7.1	3.3	3.1	2.0	3.4	2.3	0.9	12.5	14.9	2.6	2.1
	KIZ 2013000261	29.8	13.2	9.0	11.2	6.7	3.1	2.6	1.9	3.0	2.0	0.9	12.1	16.3	2.4	2.0
	KIZ 2015000385	29.1	13.7	8.7	11.4	6.9	3.0	2.6	2.0	3.1	2.0	0.9	13.2	12.4	2.2	1.7
	KIZ 2015000372	27.4	12.7	8.3	9.7	6.8	2.6	2.5	2.1	3.0	2.0	0.9	10.8	13.8	2.2	1.6
	KIZ 2015000373	29.5	12.8	8.2	11.4	5.8	3.1	2.6	2.2	3.3	1.8	1.1	12.1	14.5	2.0	1.8
	KIZ 2015000387	25.7	12.4	7.6	9.2	6.3	2.9	2.7	1.9	2.6	1.6	0.9	11.5	11.8	2.0	1.4
	KIZ 039841	23.5	10.1	6.5	8.7	5.5	2.5	2.5	1.7	2.8	1.5	0.7	9.1	14.5	2.0	1.4
	KIZ 039840	22.5	10.0	6.5	8.0	5.4	3.0	2.3	1.9	2.5	1.7	0.7	9.0	11.7	1.7	1.5
	KIZ 040162	24.4	11.0	6.9	9.2	4.7	2.6	2.0	1.8	2.7	1.9	1.0	10.0	13.1	1.9	1.6
	KIZ 040364	24.1	10.1	6.4	8.9	5.2	2.8	2.5	1.8	2.6	1.4	0.7	9.8	12.1	2.1	1.5

Table 3 Factor loadings of the first two principal components of 14 size-adjusted morphometric characteristics of males of *Glyphoglossus huadianensis* **sp. n.** and *G. yunnanensis*. Absolute values of loading greater than 0.70 in boldface. Abbreviations defined in text.

Character	PC1	PC2
Eigenvalue	6.573	1.566
% variation	46.951	11.189
HL	**0.928**	−0.130
HD	**0.856**	0.219
TD	**0.829**	−0.168
E-S	**0.829**	
E-N	**0.797**	
IO	**0.742**	0.292
HW	**0.734**	-0.330
A-G	−0.627	0.545
IN	0.609	0.548
ED	0.597	0.332
TBL	0.585	0.421
BW	−0.511	0.258
UE	0.393	−0.330
E-T		0.464

along this axis between the new species and *G. yunnanensis* (Figure 2).

The sizecorrected values of measurements follow the normal distribution (Table S2). The results of homogeneity of variables showed that *P*-value of HD, IO, and E-N were less than 0.01, rejecting null hypothesis (Table S3). One-way ANOVA test show significant difference for 9 characters at 99% significant level (Table S4), i.e., TBL, HL, HW, HD, IO, E-S, E-N, A-G, and TD. Both Brown-Forsythe and Welch test further reveal significant difference between the new species and *G. yunnanensis* in HD, IO, and E-N (Table S5).

Genetically and morphologically, the newly identified matriline differed from the species *G.yunnanensis*. Thus, we describe the new species of the genus *Glyphoglossus* below.

3.3. Taxonomic account

***Glyphoglossus huadianensis* sp. n.** (Figures 3, 4, 5; Table 2)

Holotype: KIZA00254, an adult male, collected on 2 May 2010 from Huadianba (25°52'05" N, 100°01'05" E, 2920 m elevation, Cangshan Mountain, Dali Bai Autonomous Prefecture, Yunnan, China.

Paratypes: Twelve adult males, all from Yunnan, China: from the type locality, KIZA00253, KIZA00255–KIZA00258 collected on 2 May 2010, and KIZA00252 on 27 May 2010; from Lijiang City (26°51'18" N, 100°13'40" E), KIZ 2014005754, KIZ 2014005781–KIZ 2014005785 collected in June 2010.

Etymology: The name *huadianensis* refers to Huadianba, the

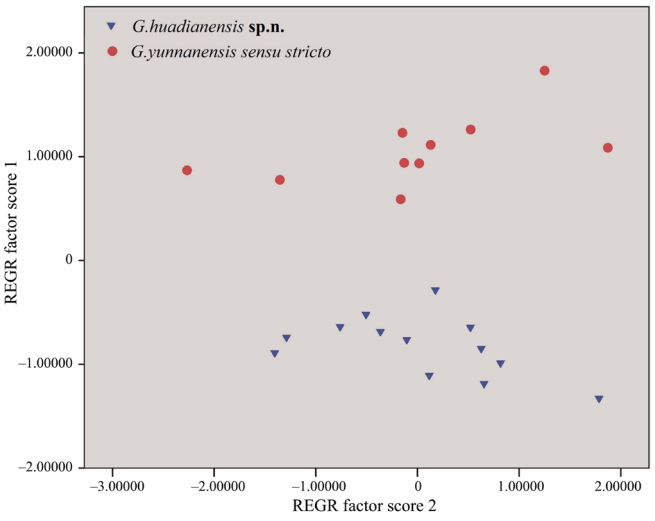

Figure 2 Scatterplot of principal components 1 and 2 of size-adjusted morphometric data for males of *G. huadianensis* **sp.n.** and *G. yunnanensis sensu stricto*.

Figure 3 Photographs of holotype of *G. huadianensis* **sp. n.** (KIZA00254) in life and its habitat, showing (A) dorsal view, (B) lateral view, (C) be in the wild, and (D) habitat. Photographs by Hong HUI.

locality where the new species was first found. Its Chinese common name is "huā diàn xiǎo xiá kǒu wā" (花甸小狭口蛙).

Diagnosis: The new species of *Glyphoglossus* is medium-sized (average SVL 36.5 mm in adult males; body size of taxa of *Glyphoglossus* ranges from 26.9 ± 3.3 mm to 61.3 ± 0.7 mm; Table 4), and shares the following combination of characters with its congeners: flattened body; wide head; short snout; small eyes; maxillary and vomerine teeth present; paired dermal folds across palate; a crescentic, inner metatarsal tubercle present (Inger, 1966; Manthey and Grossmann, 1997; Parker 1934).

The new species can be diagnosed from other congeners by the combination of the following characters: adult male body size larger than 34 mm (SVL range of 34.8–37.8 mm); pupil

rounded; supratympanic fold distinct; tympanum concealed; toe tips obtuse; subarticular tubercles prominent and rounded; webbing between toes extensive, extending up to the second subarticular tubercle on toe IV; outer metatarsal tubercle present; dorsum tuberculate; and a pair of conspicuous large round spots in groin.

Description of holotype (adult male) (measurements in mm): A medium-sized species of *Glyphoglossus*, SVL 37.3 mm; body stout ; head much broader than long (HW/HL ratio 1.42); snout rounded, projecting slightly beyond mandible in ventral view; canthus rostralis indistinct; loreal region oblique; nostrils rounded, upward positioned, slightly closer to tip of snout than to eye (E–N/E–S ratio 0.63); internarial distance slightly greater than eye to nostril distance (IN/E–N ratio 1.03); eye small (ED/HL ratio 0.38), its diameter greater than eye to nostril distance (ED/E–N ratio 1.59); interorbital distance broader than upper eyelid width (IO/UE ratio 1.26); maxillary teeth present; a feeble 'W'-shaped notch (= symphysial knob) on the anterior margin of mandible; mouth extends to posterior corner of eye; choanae visible; vomerine teeth present, positioning on two slightly sloping straight transverse ridges behind choanae; tongue oval, smooth, not nicked apically, free for approximately one third of its length; pupil rounded; tympanum concealed; single subgular vocal sac.

Forelimbs more than half of body size (20.1 mm vs 37.3 mm); fingers free of web; inner three fingers (I, II, III) with skin fringes; relative length of fingers (measurements in parentheses, in mm): III (7.6) >II (3.8) > I (3.1) > IV (2.6); finger tips blunt; nuptial pads absent on fingers; subarticular tubercles prominent and rounded, subarticular tubercle formula: 1:1:2:2; three metacarpal tubercles, distinct blade-like inner metacarpal tubercle longer than the outer, median smallest.

Hind limbs short (TBL/SVL ratio 0.42), and tibiotarsal articulation reaches up to angle of the jaw when extended forward along the body; heels untouched each other when legs at right angle to body; dorsal surfaces of thigh and tibia tuberculate; relative length of toes: IV > III > V > II > I; toes obtuse; webbing between toes extensive, webbing formula: I 0–1 II 0–1 III 0–1 IV 1–0 V, and the absent-web sides of phalanges have dermal fringe; subarticular tubercles distinct and rounded, subarticular tubercle formula: 1:1:2:2:2; a large (~ 3.2 mm), crescentic inner metatarsal tubercle, that is longer than Toe I (2.0 mm), and an outer metatarsal tubercle small and compressed.

Table 4 Mensural and meristic data for *Glyphoglossus huadianensis* sp.n., compared with congeneric species (adapted from Das *et al.* (2014) based on published descriptions of currently recognized species of *Glyphoglossus*). Characters: 1. Male SVL (maximum, unless range given, in mm); 2. Female SVL (maximum, unless range given, in mm); 3. Supratympanic fold indistinct (0) or distinct (1); 4. Dorsum smooth or granular (0) or tuberculate (1); 5. Foot webbing absent (0), basal (1) or extensive (2); 6. Lateral fringes on toes absent (0) or present (1); 7. Outer metatarsal tubercle absent (0) or present (1); 8. Pupil shape vertical (0), horizontal (1) or rounded (2); 9. Tips of toes obtusely rounded (0) or expanded (1); 10. Interorbital fold absent (0), indistinct (1) or distinct (2); 11. Dorsum ground colour olive (0), yellow (1) or brown (2); 12. Dorsum unpatterned (0), with small spots (1) or large dark central area (2); 13. Flanks unpatterned (0), with dark blotches (1) or with red bars (2). In addition, '?' denotes an unknown character state; '/' denotes a multistate character; * denotes unknown sex; # denotes there is no sexual dimorphism in terms of size and shape for *G. molossus*.

Characters	*G. brooksii*	*G. capsus*	*G. flavus*	*G. guttulatus*	*G. minutus*	*G. smithi*	*G. volzi*	*G. yunnanensis*	*G. molossus*	*G. huadianensis* **sp.n.**
site	western Sarawak, Borneo	western Sarawak, Borneo	northern Sarawak, Borneo	Myanmar, Thailand, Laos, Vietnam, Cambodia, Peninsular Malaysia	Peninsular Malaysia	northern Sarawak, Sabah, Borneo	Sumatra	southwest China, Vietnam	North-central Myanmar, Thailand, Laos, Southern Vietnam, Cambodia, peninsular Malaysia	Dali Bai Autonomous Prefecture, and Lijiang city, Yunnan, China
1	51.0–55.0	34.2–36.0	35.3*	34.0–45.0	30.9–32.7	33.0	31.3–34.0	26.9 ± 3.3 (22.5–32.7)	61.3 ± 0.7 (41.0–94.9)#	36.5 ± 0.9 (34.8–37.8)
2	60.0–73.5	?	?	38.0–50.0	25.5	37.0–39.0	31.3(34.0*)	40.0–48.8		?
3	0	0	0	1	1	1	1	1	1	1
4	1	0	0	0	1	0	1	0	0	1
5	1	1	1	1	2	1	2	2	2	2
6	1	1	1	1	1	?	1	1	?	1
7	0	1	0	0	1	0	0	1	1	1
8	0	2	?	0	2	1/2	1/2	2	0	2
9	0	0	1	0	0	1	1	0	0	0
10	0	1	1	1	0	?	2	0	1	0
11	1	0	1	0	1	2	2	2	0	2
12	1	1	1	2	2	2	1	2	0	0/1
13	1	2	0	0	0	1	1	0	0	0

Dorsum covered with slender tubercles or granules edged with black border, also small granules scattered over the head, and limbs; an indistinct and interrupted dark lateral band from the posterior of eye to middle of torso, which extend forward to canthus rostralis; supratympanic fold extending from posterior-most point of eye to angle of jaws; abdomen and inner side of limbs smooth.

Color of holotype in life: Dorsal surface yellowish-brown; supratympanic fold with an interrupted pale-edged dark line; loreal and tympanic region pale yellowish-brown, mottled with dark brown spots or patches on edge of upper jaw; flanks light yellow, mottled with brown patches; reticular markings on posterior of thighs and region around vent with a yellow background and brown border; one or two indistinct dark stripes on fore limb and tibia dorsally; a pair of conspicuous large round black spots in groin (4.6 mm × 3.7 mm), with yellow

Figure 4 Views of holotype of *G. huadianensis* **sp. n.** (KIZA00254) in preservative: dorsal (A), ventral view (B), lateral (C), posterior of thighs and region around vent (D), tympanum (E), mandible (F), vomerine teeth (G), features of dorsal skin (H), ventral view of hand (I) and foot (J).

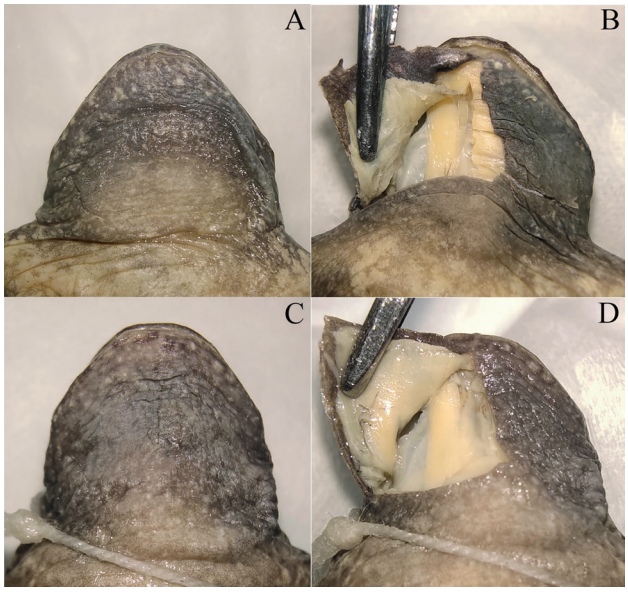

Figure 5 Vocal sac view of *G. huadianensis* **sp. n.** and *G. yunnanensis:* ventral view of gular skin (A) and anatomical structure of vocal sac (B) of *G. yunnanensis;* ventral view of gular skin (C) and anatomical structure of vocal sac (D) of *G. huadianensis* **sp. n.**

border.

Variations: Morphometric variation of the type series is shown in Table 2. Because type series are all males, sexual dimorphism of the new species could not be determined. Paratypes are generally similar to the holotype in body proportions, with only slight variation observed in three aspects. Firstly and most obviously, specimens from the type locality are differentiated from those from Lijiang City in the following respects (Figure S1): dorsal colouration (yellowish-brown *vs.* earth yellow); shape of the dorsal pattern (irregular marking *vs.* regular dotted stripes); pattern of posterior side of thighs and region around vent (with reticular markings *vs.* no reticular markings); and webbing formula: (I 0–1 II 0–1 III 0–1 IV 1–0 V *vs.* I 0–1 II 0–1 III 0–1^{-} IV 1^{-}–0 V). Also, there is 0.1% genetic divergence (p-distance, based on COI gene, Genebank accession number in Table S1) between these two populations. Secondly, there are only two metacarpal tubercles in four paratypes (KIZ 2014005781, KIZ 2014005784, KIZ 2014005754, KIZA00255). Thirdly, the subarticular tubercle formula of three paratypes (KIZA00252, KIZA00253, KIZA00256) is: 1:1:2:3:2.

Distribution and ecology: So far, the new species is known from two localities: the type locality, Huadianba, Cangshan Mountain, Dali Bai Autonomous Prefecture, Yunnan Province, and Lijiang City, Yunnan Province. At the type locality, tracked their call, the new species was found in puddles in meadows (Figure 3). No females, tadpoles or eggs were found. *Rana shuchinae* Liu, 1950 was also encountered at the type locality.

Comparisons: The new species, *Glyphoglossus huadianensis* sp. n., has geographically and genetically closer relationship with *G. yunnanensis* than with other known congeners according to recent work (Zhang *et al.*, 2020), but morphologically it can be significantly differentiated from *G. yunnanensis* ($P < 0.01$) based on one-way ANOVA test (Tables S4, S5): larger body size, SVL of 36.5 ± 0.9 mm (34.8–37.8 mm) in adult males ($n = 13$) (*vs.* 26.9 ± 3.3 mm (22.5–32.7 mm) in *G. yunnanensis*, $n = 10$); smaller ratios of head length (HL) (0.24 *vs.* 0.29), head width (HW) (0.34 *vs.* 0.37), head depth (HD) (0.19 *vs.* 0.22), interorbital distance (IO) (0.08 *vs.* 0.09), E-S (0.09 *vs.* 0.11), E-N (0.06 *vs.* 0.07), tibia length (TBL) (0.43 *vs.* 0.45), tympanum diameter (TD) (0.06 *vs.* 0.08) to body size (SVL); larger ratio of A-G (0.45 *vs.* 0.41) to body size (SVL). Also, *G. huadianensis* **sp. n.** differs from *G. yunnanensis* by having tuberculate dorsum (*vs.* dorsum smooth, except for some feebly raised glandular ridges which correspond with the outlines of the markings), having irregular markings/regular dotted stripe on dorsum (*vs.* dorsum with a symmetrical vase-shaped brown marking, edged with darker and lighter, from between the eyes to the sacral region), having an indistinct and interrupted dark lateral band from the posterior of eye to middle of torso (*vs.* a very distinct dark lateral band from the eye to the groin, bordered above by a series of more or less confluent black spots), having indistinct canthus rostralis (*vs.* canthus rostralis distinct), having prominent subarticular tubercles (*vs.* subarticular tubercles feeble prominent), having heels untouched each other when legs at right angle to body (*vs.* heels feebly overlapping).

The new species from Yunnan Province, western China differs from all other congeners in Southeast Asia in morphology (Table 4). *Glyphoglossus brooksii* (Boulenger, 1904), a Bornean endemic, distributed in western Sarawak (Malaysia), north-central and West Kalimantan (Indonesia) (IUCN SSC Amphibian Specialist Group 2019, and references therein). The new species *G. huadianensis* sp. n. can be distinguished from *G. brooksii* by the following characters: smaller SVL of adult males 36.5 ± 0.9 mm (34.8–37.8 mm) (*vs.* 51–55 mm in *G. brooksii*: Inger, 1966; Parker, 1934); supratympanic fold distinct (*vs.* indistinct); foot webbing extensive (*vs.* basal); outer metatarsal tubercle present (*vs.* absent); pupil round (*vs.* vertically elliptical); dorsum yellow-brown/earth yellow and no spots on the sides of the body (*vs.* dorsum yellow, with two dark stripes or rows of spots, and flanks with small black blotches).

Glyphoglossus flavus (Kiew, 1984) is distributed in Borneo, Gunung Mulu National Park (Sarawak), Danum Valley Conservation Area (Sabah), and Ulu Temburon National Park (Brunei) (IUCN SSC Amphibian Specialist Group, 2018, and references therein); *G. huadianensis* sp. n. differs from *G. flavus* by dorsum tuberculate (*vs.* smooth); foot webbing extensive (*vs.* basal); outer metatarsal tubercle present (*vs.* absent); tips of toes obtusely rounded (*vs.* expanded); dorsum yellow-brown/earth yellow and flanks without stripe (*vs.* orange-yellow, and flanks

with black stripes).

Glyphoglossus guttulatus (Blyth, 1856) is distributed in Southern Myanmar, most of mainland Thailand, the Central Highlands of Vietnam, Laos and south-western Cambodia (Frost, 2020); *G. huadianensis* sp. n. differs from *G. guttulatus* in the following characters: smaller SVL of adult males 36.5 ± 0.9 mm (34.8–37.8 mm) (*vs.* 34.0–45.0 mm in *G. guttulatus*: Manthey and Grossmann, 1997); foot webbing extensive, extending up to the second subarticular tubercle on toe IV (*vs.* basally webbed); outer metatarsal tubercle present (*vs.* absent); pupil round (*vs.* vertical); dorsum tuberculate (*vs.* dorsum smooth with distinct black-edged brown irregular markings).

Glyphoglossus minutus (Das *et al.*, 2004) is distributed in Peninsular Malaysia; *G. huadianensis* sp. n. differs from *G. minutus* in the following characters: larger SVL of adult males 36.5 ± 0.9 mm (34.8–37.8 mm) (*vs.* 30.9–32.7 mm in *G. minutus*); irregular markings/regular dotted stripe on dorsum (*vs.* a triangular mark, wide anteriorly in interorbital region, and a darker variegation and a large, dark, central area on dorsum); tympanum concealed (*vs.* present); loreal region oblique (*vs.* vertical); finger tips blunt (*vs.* pointed); relative length of fingers: III >II > I > IV (*vs.* III > II > IV > I); relative length of toes: IV > III > V > II > I (*vs.* IV > V > III > II > I).

Glyphoglossus smithi (Barbour and Noble, 1916) occurs in northern Sarawak and Sabah, Malaysia, and Sumatra, Indonesia; *G. huadianensis* sp. n. differs from *G. smithi* in the following characters: larger SVL of adult males 36.5 ± 0.9 mm (34.8–37.8 mm) (*vs.* 33.0 mm in *G. smithi*: Manthey and Grossmann, 1997); foot webbing extensive (*vs.* basally webbed); outer metatarsal tubercle present (*vs.* absent); toe tips obtusely rounded (*vs.* expanded); dorsum yellow-brown/earth yellow and tuberculate (*vs.* dorsum smooth and dark brownish grey); flanks without spots (*vs.* flanks with symmetrical black blotches, edged with pinkish); reticular markings around vent (*vs.* a red X-shaped spot above the vent).

Glyphoglossus volzi (Van Kampen, 1905) occurs in north-western and south-eastern Sumatra, Indonesia; *G. huadianensis* sp. n. differs from *G. volzi* in the following characters: larger SVL of adult males 36.5 ± 0.9 mm (34.8–37.8 mm) (*vs.* 31.3–34.0 mm in *G. volzi*); outer metatarsal tubercle present (*vs.* absent); toe tips obtusely rounded (*vs.* expanded); interorbital dermal fold absent (*vs.* distinct); dorsum yellow-brown/earth yellow and flanks without spots (*vs.* dorsum reddish-brown with black spots, and flanks with dark blotches).

Glyphoglossus capsus (Das *et al.*, 2014) is distributed in Matang Range in Sarawak, Malaysia, and the upper reaches of Gunung Penrissen (the boundary between Sarawak (Malaysia) and Kalimantan (Indonesia)). *G. huadianensis* sp. n. differs from *G. capsus* in the following characters: supratympanic fold distinct (*vs.* indistinct); foot webbing extensive (*vs.* basally webbed); dorsum yellow-brown/earth yellow and flanks without spots (*vs.* dorsum olive with red spots and venter with a large area of red).

Glyphoglossus molossus Günther, 1869, a large, globose frog, is found in north-central Myanmar, most of mainland Thailand, Laos, and southern Vietnam; *G. huadianensis* sp. n. differs from *G. molossus* in the following characters: smaller SVL of adult males 36.5 ± 0.9 mm (34.8–37.8 mm) (*vs.* SVL 61.3 ± 0.7 mm (41.0–94.9 mm) in *G. molossus*, Laojumpon *et al.* 2012); dorsum yellow-brown/earth yellow and tuberculate (*vs.* dorsum brownish olive and smooth, with large dark central area); pupil round (*vs.* vertical); snout rounded (*vs.* an unusually truncate snout: snout very short and blunt covered by a leathery finely granular skin; the fleshy part of the lower jaw swollen, truncated in front, forming a semicircular disk).

An additional species has been described in the genus-*Calluella ocellata* Liu (1950), from "Szekuaipa, Chaochiaohsien, Sikang, 7800 feet" (currently, Sikuaiba, Zhaojue County, Sichuan Province, China). The study of Zhang *et al.* (2020) included samples from Yanziluo village, Jiefang township, Zhaojue County, Sichuan Province, China, 3129 m, and discovered the morphological characteristics of these specimens were consistent with the original description of *Calluella ocellata*, but they clustered together (member of lineage C), and were nested among specimens of *G. yunnanensis*. That is to say, the results supported Liu and Hu (1961): *Calluella ocellata* is a synonymy of *G. yunnanensis* Boulenger 1919. Moreover, *G. huadianensis* sp. n. (lineage LD) was deeply separated from lineages C with a divergence of 9.2% (Zhang *et al.*, 2020). So, no further morphological comparison was conducted.

4. Discussion

Zhang *et al.* (2020) investigated the geographical distribution and pattern of genetic variation of *G. yunnanensis*, and discovered one of maternal lineages (LD) is deeply divergent from the remaining ones (W, C, E) (Figure 1), suggested it may be an undescribed species. In the study, morphological comparisons and genetic divergence clearly support this possibility and demonstrate that the lineage LD is a new species, named *G. huadianensis* sp. n., and is distinctive from *G. yunnanensis* and other known congeners. Firstly, based on 2154 bp concatenated mitochondrial gene fragments, the genetic distance between *G. huadianensis* sp. n. and the *G. yunnanensis sensu stricto* (lineages W+C+E) was 8.9%, and the difference between *G. huadianensis* sp. n. and other known congeners was 7.8%–20.0% (Table 1). Secondly, the result of PCA and ANOVA indicates that *G. huadianensis* sp. n. differs from *G. yunnanensis* significantly. As well, *G. huadianensis* sp. n. separated from other known congeners by a series of morphometric characters

(Table 4). One main factor probably hindered the discovery of the new species described here: there are obvious intraspecific variation in the color pattern and body size among populations of *G. yunnanensis* (personal observation). *G. huadianensis* sp. n. was superficially morphologically similar with specimens from some populations of *G. yunnanensis*, easily misleading the identifications if made without detailed morphological and genetic examination. In future investigations, data on advertisement calls and larvae may be particularly helpful in resolving the taxonomic uncertainty.

In addition to *G. yunnanensis*, the new taxa described herein is the second species known from Yunnan, China in the genus *Glyphoglossus*. The discovery of the new species indicates that the diversification of *Glyphoglossus* in China still needs further investigation. Moreover, the new species and *G. yunnanensis* were found to co-distribute in Dali Bai Autonomous Prefecture and Lijiang City, which suggests that these regions may play an important role in the formation and evolution of species diversity of *Glyphoglossus*.

Among specimens of *G. huadianensis* sp. n., distinct morphological variation occurs between populations Dali and Lijiang in dorsal colouration, shape of the dorsal pattern, pattern of posterior side of thighs and region around vent, and webbing formula. But the genetic distance between them was 0.1%, which was much less than interspecific differentiation. These evidences indicated variation between them are only intraspecific differentiation.

To date, the new species was only found in Dali Bai Autonomous Prefecture and Lijiang City, and more research is needed on its life history information and distribution to determine its conservation level.

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Appendix

Table S1 Information on Voucher No., GenBank No., and localities of specimens used in this study.

Species	Voucher	Locality data	GenBank No.		
			16S	COI	CYTB
Outgroups					
G. minuta	KUHE:52463	Malaysia: Pahang, Temerloh	AB5983402	N/A	N/A
G. guttulata	FMNH 252955	Vietnam: Gia-Lai Province: Ankhe District	DQ2831443	KM5097684	N/A
G. molossus	CAS 210056	Myanmar: Sagaing	KM5091354	KM5097984	AB2012251
Microhyla ornata			NC_009422	NC_009422	NC_009422
Microhyla okinavensis			NC_010233	NC_010233	NC_010233
Microhyla heymonsi			NC_006406	NC_006406	NC_006406
Microhyla pulchra			KX021976	KX021976	KX021976
Lineage LD					
G. huadianensis **sp. n.**	2014005754	China: Yunnan, Lijiang	N/A	MN851525	MN851942
G. huadianensis **sp. n.**	2014005781	China: Yunnan, Lijiang	MN860396	MN851551	MN851968
G. huadianensis **sp. n.**	2014005782	China: Yunnan, Lijiang	N/A	MN851552	MN851969
G. huadianensis **sp. n.**	KIZA00253	China: Yunnan, Dali	N/A	MN851676	N/A
G. huadianensis **sp. n.**	KIZA00254	China: Yunnan, Dali	N/A	MN851677	N/A
G. huadianensis **sp. n.**	KIZA00255	China: Yunnan, Dali	N/A	MN851678	N/A
G. huadianensis **sp. n.**	KIZA00256	China: Yunnan, Dali	N/A	MN851679	N/A
G. huadianensis **sp. n.**	KIZA00257	China: Yunnan, Dali	N/A	MN851680	N/A
G. huadianensis **sp. n.**	KIZA00258	China: Yunnan, Dali	N/A	MN851681	N/A
G. huadianensis **sp. n.**	KIAZ00252	China: Yunnan, Dali	N/A	MN851675	MN852092
***G. yunnanensis sensu stricto* (lineage W+C+E)**					
	2014003016	China: Yunnan, Yongde, Wumulong	MN860350	MN851441	MN851858
	2014003020	China: Yunnan, Yongde, Wumulong	MN860353	MN851445	MN851862
	2014003019	China: Yunnan, Yongde, Wumulong	MN860352	MN851444	MN851861
	2014003017	China: Yunnan, Yongde, Wumulong	MN860351	MN851442	MN851859
	2014003008	China: Yunnan, Cangyuan, Danjia	MN860349	MN851434	MN851851
	2016000182	China: Yunnan, Shidian, Xunyang	MN860435	MN851660	MN852077
	2016000188	China: Yunnan, Shidian, Xunyang	MN860440	MN851666	MN852083
	2014003103	China: Yunnan, Changning	MN860378	MN851505	MN851922
	2014003025	China: Yunnan, Yongde, Wumulong	MN860355	MN851448	MN851865
	2013000209	China: Yunnan, Baoshan, Lujiang	MN860295	MN851315	MN851732
	2013000210	China: Yunnan, Baoshan, Lujiang	MN860296	MN851316	MN851733
	2016000181	China: Yunnan, Shidian, Xunyang	MN860434	MN851659	MN852076
	2016000192	China: Yunnan, Shidian, Xunyang	MN860444	MN851670	MN852087
	2014003051	China: Yunnan, Lianghe, Xiaochang	MN860362	MN851465	MN851882
	2015000868	China: Yunnan, Dayao, Santai	MN860426	MN851631	MN852048
	2015000873	China: Yunnan, Dayao, Santai	MN860427	MN851634	MN852051
	2015000699	China: Sichuan, Yanyuan, Baiwu	MN860404	MN851586	MN852003
	2015000707	China: Sichuan, Huidong, Lunan	MN860407	MN851594	MN852011
	2015000859	China: Yunnan, Wuding, Chadian	MN860419	MN851623	MN852040
	2015000727	China: Yunnan, Lyuchun, Gekui	MN860413	MN851613	MN852030

(Continued Table S1)

Species	Voucher	Locality data	GenBank No.		
			16S	COI	CYTB
	2015000854	China: Yunnan, Wuding, Chadian	MN860414	MN851618	MN852035
	2015000861	China: Yunnan, Wuding, Chadian	MN860421	MN851625	MN852042
	2015000871	China: Yunnan, Dayao, Santai	N/A	MN851632	MN852049
	2015000855	China: Yunnan, Wuding, Chadian	MN860415	MN851619	MN852036
	WD20150626001	China: Yunnan, Wuding, Shishan	MN860455	MN851691	MN852102
	2015000857	China: Yunnan, Wuding, Chadian	MN860417	MN851621	MN852038
	2015000863	China: Yunnan, Wuding, Chadian	MN860423	MN851627	MN852044
	2015000860	China: Yunnan, Wuding, Chadian	MN860420	MN851624	MN852041
	2015000862	China: Yunnan, Wuding, Chadian	MN860422	MN851626	MN852043
	No voucher	China: Yunnan, Zhaoyang, Sujiaxiang	N/A	N/A	N/A
	2014000263	China: Guizhou, Weining, Xueshan	MN860315	MN851353	MN851770
	2014000324	China: Yunnan, Huize, Wuxing	MN860320	MN851364	MN851781
	2014005764	China: Sichuan, Zhaojue, Jiefangxiang	MN860391	MN851534	MN851951
	YX150618008	China: Sichuan, Yuexi, Puxiong	MN860465	MN851709	MN852120
	2015000703	China: Sichuan, Huidong, Lunan	MN860405	MN851590	MN852007
	2014005760	China: Sichuan, Zhaojue, Jiefangxiang	MN860390	MN851530	MN851947
	2014003101	China: Yunnan, Fengqing, Xinhua	MN860376	MN851503	MN851920
	2014003112	China: Yunnan, Changning	MN860382	MN851513	MN851930
	2014000550	China: Yunnan, Lanping, Yingpan	MN860341	MN851416	MN851833
	2014000547	China: Yunnan, Lanping, Yingpan	MN860338	MN851413	MN851830
	2016000184	China: Yunnan, Shidian, Xunyang	MN860437	MN851662	MN852079
	2014000548	China: Yunnan, Lanping, Yingpan	MN860339	MN851414	MN851831
	2014000549	China: Yunnan, Lanping, Yingpan	MN860340	MN851415	MN851832
	2014002992	China: Yunnan, Yunxian, Yongbao	MN860343	MN851420	MN851837
	2014003001	China: Yunnan, Yunxian, Yongbao	MN860346	MN851428	MN851845
	2014002995	China: Yunnan, Yunxian, Yongbao	MN860344	MN851423	MN851840
	2014002991	China: Yunnan, Yunxian, Yongbao	MN860342	MN851419	MN851836
	2016000159	China: Yunnan, Jingdong, Taizhong	MN860429	MN851641	MN852058
	2016000162	China: Yunnan, Jingdong, Taizhong	MN860430	MN851642	MN852059
	2015000872	China: Yunnan, Dayao, Santai	N/A	MN851633	MN852050
	2014003039	China: Yunnan, Linxiang, Daxueshan	MN860359	MN851459	MN851876
	2013000396	China: Yunnan, Jingdong, Jingpin	MN860304	MN851329	MN851746
	2013000400	China: Yunnan, Jingdong, Jingpin	MN860308	MN851333	MN851750
	2014003102	China: Yunnan, Dali, Binchuan	MN860377	MN851504	MN851921
	2014003072	China: Yunnan, Linxiang, Boshang	MN860369	MN851481	MN851898
	2014003077	China: Yunnan, Linxiang, Boshang	MN860371	MN851485	MN851902
	2014003062	China: Yunnan, Linxiang, Boshang	MN860367	MN851473	MN851890
	2014003100	China: Yunnan, Fengqing, Xinhua	MN860375	MN851502	MN851919
	2015000877	China: Yunnan, Yongping, Shuixie	MN860428	MN851638	MN852055
	2016000172	China: Yunnan, Chuxiong, Xishelu	MN860431	MN851650	MN852067
	2013000397	China: Yunnan, Jingdong, Jingpin	MN860305	MN851330	MN851747
	2014002996	China: Yunnan, Yunxian, Yongbao	MN860345	MN851424	MN851841

(Continued Table S1)

Species	Voucher	Locality data	GenBank No.		
			16S	COI	CYTB
	2013000381	China: Yunnan, Menghai, Xiding	MN860298	MN851318	MN851735
	2013000384	China: Yunnan, Menghai, Xiding	MN860301	MN851321	MN851738
	2014003087	China: Yunnan, Cangyuan, Mengjiao	MN860372	MN851491	MN851908
	2014003005	China: Yunnan, Cangyuan, Danjia	MN860348	MN851432	MN851849
	2014000452	China: Yunnan, Huize, Dahai	MN860331	MN851390	MN851807
	2014000442	China: Yunnan, Huize, Dahai	MN860328	MN851380	MN851797
	2014000499	China: Yunnan, Fuyuan, Huangnihe	MN860336	MN851407	MN851824
	2014000500	China: Yunnan, Fuyuan, Huangnihe	MN860337	MN851408	MN851825
	2014000489	China: Yunnan, Fuyuan, Huangnihe	MN860332	MN851397	MN851814
	2015000721	China: Yunnan, Mile, Dongshan	MN860411	MN851608	MN852025
	2015000713	China: Yunnan, Mile, Dongshan	MN860409	MN851600	MN852017
	2015000723	China: Yunnan, Mile, Dongshan	MN860412	MN851610	MN852027
	2014000408	China: Yunnan, Zhanyi, Yanfang	MN860321	MN851365	MN851782
	2014000415	China: Yunnan, Fuyuan, Mohong	MN860324	MN851368	MN851785
	WD20150626006	China: Yunnan, Wuding, Shishan	N/A	MN851696	MN852107
	2014000418	China: Yunnan, Fuyuan, Mohong	MN860326	MN851370	MN851787
	2014005756	China: Yunnan, Xuanwei, Dongshan	MN860388	MN851527	MN851944
	2015000712	China: Yunnan, Mile, Dongshan	MN860408	MN851599	MN852016
	2014000447	China: Yunnan, Huize, Dahai	MN860329	MN851385	MN851802
	WD20150626012	China: Yunnan, Wuding, Shishan	MN860463	MN851701	MN852112
	WD20150626009	China: Yunnan, Wuding, Shishan	MN860461	MN851699	MN852110
	2013000316	China: Yunnan, Anning, Bajie	MN860297	MN851317	MN851734
	TH1407082A	China: Yunnan, Tonghai, Lishan	MN860452	MN851685	MN852096
	2015000377	China: Yunnan, Kunming, Xishanqu	MN860398	MN851560	MN851977
	2015000865	China: Yunnan, Wuding, Chadian	MN860425	MN851629	MN852046
	2015000372	China: Yunnan, Kunming, Xishanqu	MN860397	MN851555	MN851972
	WD20150626004	China: Yunnan, Wuding, Shishan	MN860457	MN851694	MN852105
	TH1407085A	China: Yunnan, Tonghai, Lishan	MN860453	MN851687	MN852098
	DC130721001	China: Yunnan, Dongchuan, Hongtudi	MN860449	MN851674	MN852091
	TH1407080A	China: Yunnan, Tonghai, Lishan	MN860450	MN851683	MN852094
	TH1407081A	China: Yunnan, Tonghai, Lishan	MN860451	MN851684	MN852095
	TH1407088A	China: Yunnan, Tonghai, Lishan	MN860454	MN851690	MN852101
	2015000384	China: Yunnan, Kunming, Xishanqu	MN860399	MN851565	MN851982
	2014000248	China: Guizhou, Weining, Xueshan	MN860310	MN851339	MN851756
	2015000691	China: Yunnan, Yiliang, Longjie	MN860403	MN851579	MN851996
	20110620106	China: Yunnan, Yunnan, Lijiang	MN860293	MN851308	MN851725
	2014000283	China: Guizhou, Shuicheng,Yushe	MN860316	MN851356	MN851773
	2014000250	China: Guizhou, Weining, Xueshan	MN860312	MN851341	MN851758
	2014005773	China: Yunnan, Zhenxiong, Wanchang	MN860393	MN851543	MN851960
	2014005780	China: Yunnan, Zhenxiong, Wanchang	MN860395	MN851550	MN851967
	2014000287	China: Guizhou, Shuicheng, Yushe	MN860319	MN851360	MN851777
	2014005777	China: Yunnan, Zhenxiong, Wanchang	MN860394	MN851547	MN851964
	2014000285	China: Guizhou, Shuicheng, Yushe	MN860318	MN851358	MN851775

(Continued Table S1)

Species	Voucher	Locality data	GenBank No.		
			16S	COI	CYTB
	2015000682	China: Yunnan, Yiliang, Longjie	MN860401	MN851570	MN851987
	2015000705	China: Sichuan, Huidong, Lunan	MN860406	MN851592	MN852009
	2011062092	China: Yunnan, Yunnan, Lijiang	MN860294	MN851314	MN851731
	20110620100	China: Yunnan, Yunnan, Lijiang	MN860291	MN851304	MN851721
	ZTSJ140730001	China: Yunnan, Zhaoyang, Sujiaxiang	MN860466	MN851713	MN852124
	2011062075	China: Yunnan, Yunnan, Lijiang	N/A	MN851310	MN851727
	2011062083	China: Yunnan, Yunnan, Lijiang	N/A	MN851312	MN851729
	2011062076	China: Yunnan, Yunnan, Lijiang	N/A	MN851311	MN851728
	ZTSJ140730003	China: Yunnan, Zhaoyang, Sujiaxiang	N/A	MN851715	MN852126
	2011062087	China: Yunnan, Yunnan, Lijiang	N/A	MN851313	MN851730
	ZTSJ140730002	China: Yunnan, Zhaoyang, Sujiaxiang	N/A	MN851714	MN852125

Table S2 Test of Normality based on Shapiro-Wilk Test ($P < 0.01$).

	G. huadianensis	*G. yunnanensis*
TBL	0.041	0.815
HL	0.740	0.690
HW	0.338	0.703
HD	0.645	0.291
ED	0.260	0.099
IO	0.507	0.248
IN	0.990	0.632
E-S	0.348	0.658
E-N	0.057	0.512
E-T	0.962	0.410
A-G	0.163	0.079
BW	0.239	0.837
TD	0.202	0.855
UE	0.770	0.906

Table S3 Test of Homogeneity of Variances ($P < 0.01$).

	Levene Statistic	*df*1	*df*2	Significance
TBL	3.994	1	21	0.059
HL	1.992	1	21	0.173
HW	0.476	1	21	0.498
HD	9.669	1	21	0.005*
ED	1.297	1	21	0.268
IO	14.630	1	21	0.001*
IN	2.997	1	21	0.098
E-S	0.134	1	21	0.718
E-N	9.028	1	21	0.007*
E-T	0.008	1	21	0.930
A-G	2.509	1	21	0.128
BW	0.162	1	21	0.692
TD	0.473	1	21	0.499
UE	0.026	1	21	0.873

Table S4 Analysis of variance (ANOVA) at one way.

		Sum of squares	*df*	Mean square	*F*	Significance
TBL	Between Groups	0.003	1	0.003	9.905	0.005
	Within Groups	0.005	21	0.000		
	Totle	0.008	22			
HL	Between Groups	0.013	1	0.013	123.951	0.000
	Within Groups	0.002	21	0.000		
	Totle	0.015	22			
HW	Between Groups	0.004	1	0.004	28.649	0.000
	Within Groups	0.003	21	0.000		
	Totle	0.007	22			
HD	Between Groups	0.008	1	0.008	39.781	0.000
	Within Groups	0.004	21	0.000		
	Totle	0.013	22			
ED	Between Groups	0.000	1	0.000	6.812	0.016
	Within Groups	0.001	21	0.000		
	Totle	0.002	22			
IO	Between Groups	0.001	1	0.001	25.393	0.000
	Within Groups	0.001	21	0.000		
	Totle	0.002	22			
IN	Between Groups	0.000	1	0.000	6.842	0.016
	Within Groups	0.001	21	0.000		
	Totle	0.001	22			
E-S	Between Groups	0.001	1	0.001	33.059	0.000
	Within Groups	0.001	21	0.000		
	Totle	0.002	22			
E-N	Between Groups	0.000	1	0.000	20.770	0.000
	Within Groups	0.000	21	0.000		
	Totle	0.001	22			
E-T	Between Groups	0.000	1	0.000	0.081	0.778
	Within Groups	0.000	21	0.000		
	Totle	0.000	22			
A-G	Between Groups	0.009	1	0.009	10.812	0.004
	Within Groups	0.017	21	0.001		
	Totle	0.025	22			
BW	Between Groups	0.017	1	0.017	7.811	0.011
	Within Groups	0.046	21	0.002		
	Totle	0.064	22			
TD	Between Groups	0.002	1	0.002	47.123	0.000
	Within Groups	0.001	21	0.000		
	Totle	0.002	22			
UE	Between Groups	0.000	1	0.000	3.774	0.066
	Within Groups	0.000	21	0.000		
	Totle	0.000	22			

Table S5 Results of both Brown-Forsythe and Welch test.

		Statistic[a]	*df*1	*df*2	Significance
TBL	Welch	8.506	1	12.940	0.012
	Brown-Forsythe	8.506	1	12.940	0.012
HL	Welch	112.815	1	15.405	0.000
	Brown-Forsythe	112.815	1	15.405	0.000
HW	Welch	27.348	1	17.579	0.000
	Brown-Forsythe	27.348	1	17.579	0.000
HD	Welch	32.744	1	11.385	0.000
	Brown-Forsythe	32.744	1	11.385	0.000
ED	Welch	5.839	1	12.869	0.031
	Brown-Forsythe	5.839	1	12.869	0.031
IO	Welch	20.970	1	11.498	0.001
	Brown-Forsythe	20.970	1	11.498	0.001
IN	Welch	5.753	1	12.138	0.033
	Brown-Forsythe	5.753	1	12.138	0.033
E-S	Welch	31.254	1	17.141	0.000
	Brown-Forsythe	31.254	1	17.141	0.000
E-N	Welch	17.609	1	12.444	0.001
	Brown-Forsythe	17.609	1	12.444	0.001
E-T	Welch	0.082	1	19.644	0.778
	Brown-Forsythe	0.082	1	19.644	0.778
A-G	Welch	11.617	1	20.999	0.003
	Brown-Forsythe	11.617	1	20.999	0.003
BW	Welch	7.232	1	16.185	0.016
	Brown-Forsythe	7.232	1	16.185	0.016
TD	Welch	50.277	1	20.977	0.000
	Brown-Forsythe	50.277	1	20.977	0.000
UE	Welch	3.765	1	19.429	0.067
	Brown-Forsythe	3.765	1	19.429	0.067

Note: [a]: Asymptotic *F* distribution

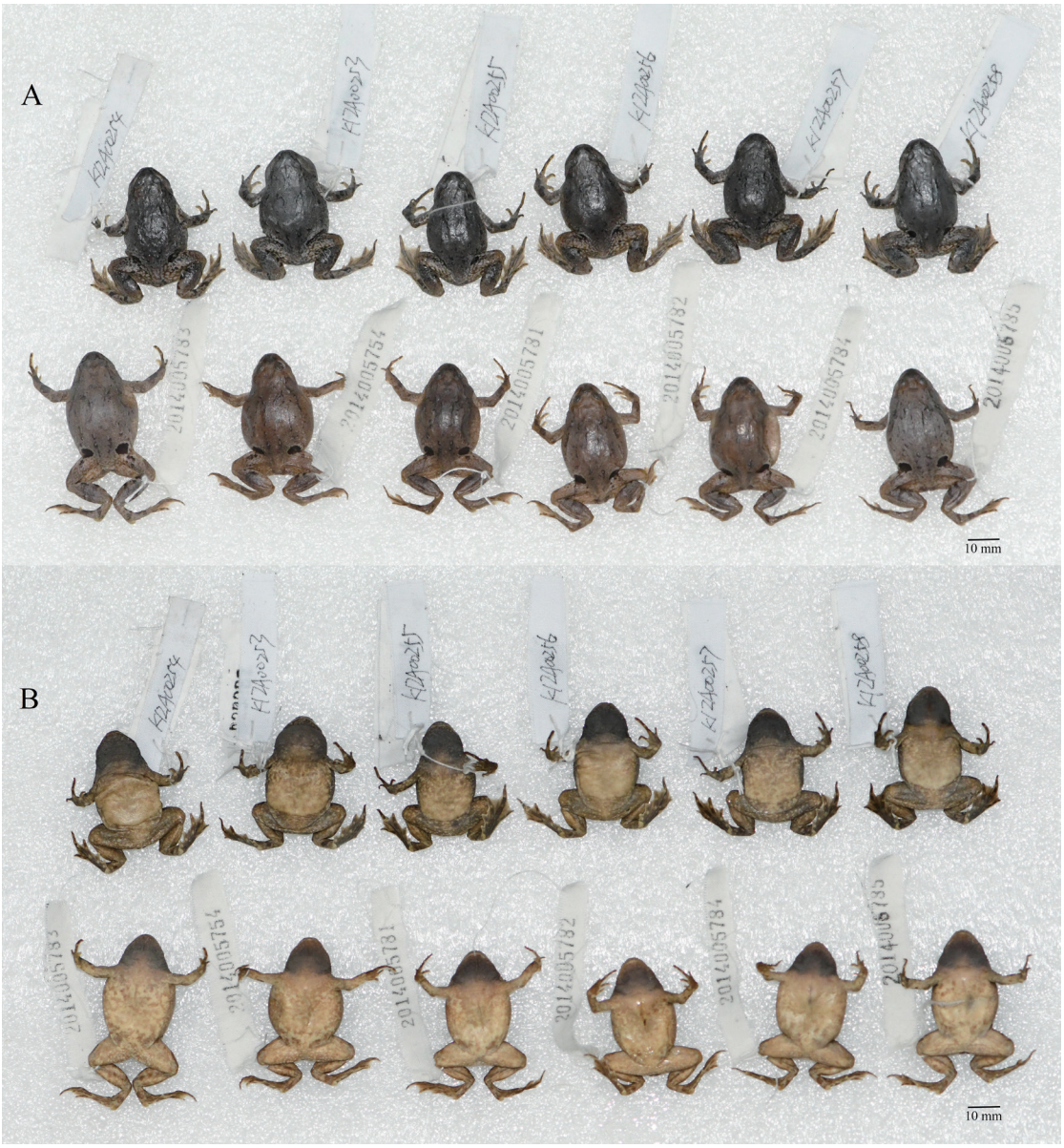

Figure S1 Photographs of individuals of *G. huadianensis* **sp. n.** from two localities: (A) dorsal view, (B) ventral view: the upper from Dali Bai Automotous Prefecture, and the lower from Lijiang City.